Brief Report

DOI: 10.5582/bst.2012.v6.3.110

Drug susceptibility pattern of *Mycobacterium tuberculosis* isolates from patients of Category-II failure of pulmonary tuberculosis under directly observed treatment short-course from north India

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Summary **The major contributing factors for the causation of treatment failure in cases of pulmonary tuberculosis under Category-II directly observed treatment short-course treatment (DOTS) are treatment after default, poor treatment compliance, and development of multi-drug resistant (MDR) tuberculosis. The objective of the present study is to find out the demographic profile and drug susceptibility pattern in Category-II failure patients of pulmonary tuberculosis under Revised National Tuberculosis Control Programme (RNTCP) of India. Two hundreds and twenty four patients with Category-II treatment failure of pulmonary tuberculosis were enrolled from Department of Pulmonary Medicine, at Chatrapati Sahuji Maharaj Medical University, UP, Lucknow, India, from August 2003 to July 2008. Their complete bacteriological assessment in terms of sputum smear for acid-fast bacilli, culture for *Mycobacterium tuberculosis* and drug sensitivity pattern were done in the Department of Microbiology. Among 224 patients, 16 (7.1%) patients were lost to follow-up and the final analysis was done among 208 (92.8%) cases. The reasons for inclusion of these 224 cases in the Category II regimen were treatment failure in the previous regimen (*n* = 75, 33%), default in 57% (*n* = 129 cases), and relapse in 8.9% (*n* = 20 cases). Among 208 patients, culture was positive in 170 (81.7%) cases, negative in 17 (8.1%) cases and contaminated in 21 (10%) cases. The drug sensitivity pattern of culture positive cases of Category-II failure patients revealed that, 58.2% (*n* = 99) had MDR tuberculosis and 40.5% (*n* = 69) were resistant but were non-MDR tuberculosis and 1.1 % (*n* = 2) cases were sensitive to all first line antituberculosis drugs.**

Keywords: Category-II, failure, MDR, tuberculosis, RNTCP

1. Introduction

India accounts for nearly one-third of the global burden of tuberculosis and two-thirds of the total cases in South-East Asia. Nearly 40 percent of the Indian population is infected with the tuberculosis bacillus. Increasing awareness of the rising global rates of multi-drug resistant (MDR) tuberculosis has led to a concerted international effort to confront this disease, particularly in India with the high incidence of tuberculosis. As per the latest estimates from the State representative drug resistance surveillance (DRS) survey of India in Gujarat states and various district level DRS studies, the prevalence of MDR tuberculosis in new smear positive

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pulmonary tuberculosis cases is < 3% and 12% to 17% among smear positive previously treated pulmonary tuberculosis cases (*1*).

In India, the Revised National Tuberculosis Control Programme (RNTCP), adopting the directly observed treatment short-course (DOTS) strategy advocated by the World Health Organization (WHO), was implemented in 1993 in the country and has been scaled up rapidly since mid 1998. In India, all RNTCP treatment regimens are given three times weekly on alternate days. Based on a stringent diagnostic algorithm and history of previous tuberculosis treatment, the diagnosed cases under RNTCP are classified as 'New' and are put on the Category-I regimen. Re-treatment cases *i.e.*, those who give a history of previous tuberculosis treatment of more than one month, are put on the Category-II regimen. This category comprises smear positive 'Failures', 'Relapses', 'Treatment after default' and 'Others'.

Under RNTCP, treatment failure is defined as, a patient who, while on treatment, remained or became again smear positive five months or later after commencing treatment. Category-II treatment failure patients, where chances of MDR tuberculosis are very high, remain the most difficult problem in the management of tuberculosis (*2,3*). In various studies, among Category-II failure patients, the prevalence of MDR tuberculosis varied from 4% to 80% (*4-8*).

In the present study we have attempted to determine the demographic profile and drug susceptibility pattern in Category-II patients of pulmonary tuberculosis, under RNTCP so that we can identify MDR tuberculosis cases early and reduce their transmission by starting second line treatment for MDR tuberculosis.

2. Materials and Methods

2.1. *Study design*

Prospective, cohort study of Category-II failure patients of pulmonary tuberculosis under RNTCP, between August 2003 and July 2008, referred from various districts of Uttar Pradesh (India) were studied.

2.2. *Setting*

The current study was set in Department of Pulmonary Medicine & Department of Microbiology, CSM Medical University, Lucknow, India (WHO recommended Intermediate Reference laboratory).

2.3. *Criteria for selection of patients*

Inclusion criteria: (*i*) All tuberculosis patients, who failed on Category-II DOTS treatment, (*ii*) Age 15-70 years. Exclusion criteria: Patients who already had taken the Category-IV regimen for treatment.

2.4. *Patients clinical evaluation*

A detailed history of previous treatment was taken and a thorough clinical examination was done with a structured questionnaire. Chest radiograph, liver and renal function tests, and blood sugar estimation were also done. Screening for human immunodeficiency virus (HIV) infection was done in all the patients after consent. The diagnosis of smear positive pulmonary tuberculosis was done as per the RNTCP diagnostic algorithm.

2.5. *Sample collection*

All patients were directed to collect the early morning sputum specimen in a sterilized wide-mouthed bottle with a tightly fitting cork stopper. Sputum was sent for smear for acid-fast bacilli (AFB) and culture/sensitivity, on three consecutive days. All sputum samples were transported to the Department of Microbiology (WHO recommended IRL laboratory), as soon as possible after collection. The Department of Microbiology has been identified as an Intermediate Reference laboratory for quality control in RNTCP under the government of India/WHO, World Bank.

2.6. *Laboratory method*

Homogenization and concentration of specimen was done by modified petroff's method and staining for acid fast bacilli was done by the Zeihl Neelsen Method as described by Cruickshank (1965) (*9*).

2.7. *Culture examination*

Conventional solid egg-based Lowenstein-Jensen (LJ) media was used for primary culture. All cultures were incubated at 35-37°C until growth was observed or discarded as negative after eight weeks. Slopes that were grossly contaminated also were discarded.

All cultures were examined 48-72 h after inoculation to detect gross contaminants and thereafter cultures were examined weekly, up to 8 weeks. The colonies of *Mycobacterium tuberculosis* were defined as rough, crumbly, waxy, non-pigmented (buff colored). Negative cultures were defined as no growth after 8 weeks. The positive cultures showing AFB were identified as *M. tuberculosis* based on the results of growth on LJ medium containing *p*-nitrobenzoic acid and niacin tests (*10,11*). The presence of AFB in primary cultures was confirmed by Zeihl Neelsen staining.

2.8. *Drug susceptibility tests*

All *M. tuberculosis* cultures were subjected to drug susceptibility tests for isoniazid (H), rifampicin (R), ethambutol (E), streptomycin (S), and pyrazinamide (Z)

by the economic version of proportion method as per the International Union against Tuberculosis and Lung Disease (IUATLD), Manual for the National Laboratory Network (*4*). The critical proportion for declaring a strain as resistant to the drugs was 1%.

2.9. *Ethical clearance*

The Ethical committee of our university approved the present study.

2.10. *Definition used in present study*

(*i*) Category-II failure: If the patient has completed more than 5 months of Category-II treatment and remained sputum positive.

(*ii*) MDR tuberculosis case: Those that have a sputum positive culture and whose tuberculosis is due to *M. tuberculosis* that are resistant *in vitro* to at least isoniazid and rifampicin (the culture and drug susceptibility test's result being from an RNTCP accredited laboratory).

(*iii*) Drug sensitive: Absence of resistance to any of the first line antituberculosis drugs, *e.g.* streptomycin, rifampicin, isoniazid, ethambutol, and pyrazinamide.

(*iv*) Monoresistance: Resistance to only 1 drug from the first line antituberculosis drugs. Polyresistance: Resistance to at least two or more drugs excluding the R and H combination.

3. Results and Discussion

A total of 224 patients, which failed on Category-II DOTS treatment, were included in the present analysis even though it was planned to collect a sputum specimen from all patients, unfortunately 16 (7.1%) patients were lost to follow-up. So the final analysis was done among, 208 (92.8%) cases. The reasons for inclusion of these 224 cases in the Category-II regimen failure cases were treatment failure in 75 cases (33%), treatment after default of previous treatment in 129 cases (57%), and relapse in 20 cases (8.9%). Among 208 patients, cultures were positive in 170 (81.7%) cases, negative in 17 (8.1%) cases, and contaminated in 21 (10%) cases. Among 170 patients, who produced positive cultures, 2 (1.1%) harbored sensitive bacilli, 99 (58.2%) had an MDR tuberculosis pattern, and 69 (40.5%) cases were resistant but a non-MDR tuberculosis pattern.

There was a preponderance of males, (n = 141, 68.2%) as compared to females (n = 67, 32.3%). Of all, 96 (46%) cases were from rural areas and 112 (54%) from urban areas. 79.4% of patients were from age group 15-35 years and from lower socioeconomic status. All patients tested were sputum smear positive for AFB and percentage grading as +3, +2, +1, and scanty were 41.7%, 27%, 23%, and 8.2%, respectively. Of all, 43 cases were current smokers and 11 of them had MDR tuberculosis and 79 cases were ex-smokers, and 38 of them had MDR tuberculosis. Fifty-seven (27.4%) cases had a history of alcohol intake and 11 of them had MDR tuberculosis. Diabetes mellitus was associated with 18 (8.6%) cases of Category-II failure and 4 of them had MDR tuberculosis. Twenty-three Category-II failure patients were infected with HIV and five of them had MDR tuberculosis.

Before Category-II treatment, 76 cases (36.5%) had taken DOTS treatment and 30 of them had MDR tuberculosis, while 132 (63.5%) cases had taken treatment from private practitioners and 69 of them had MDR tuberculosis. The detailed analysis is given in Tables 1 and 2.

One of the most important landmarks in the history of tuberculosis control is the introduction and implementation of the RNTCP based on the globally recommended DOTS strategy that was implemented in India in a phased manner since 1993. W.H.O. recommends retreatment with the Category-II Anti Tuberculosis Treatment regimen (2H3R3Z3E3S3 + 1H3R3Z3E3 + 5H3R3E3) for patients with relapse, treatment failure or treatment after interruption (*5*). The treatment success rate for previously treated patients with the re-treatment regimen, *i.e.,* Category-II was low 71%. The probable reasons for Category-II treatment failure are treatment after default, poor treatment compliance and development of MDR tuberculosis.

The present study revealed that 58.2% patients of Category-II failure had MDR tuberculosis and 40.5% cases had non-MDR tuberculosis.

Table 1. Demographic profile of MDR tuberculosis cases (n = 99/170*)

Item	Number	Percentage
Sex		
Male	65	38.2%
Female	34	20.0%
Age wise distribution		
< 15-24 years	33	19.4%
25-34 years	45	26.4%
35-45 years	20	11.7%
> 45 years	1	0.5%
Residence		
Rural	41	24.1%
Urban	58	34.1%
H/O Smoking		
Current smokers	11	6.4%
Ex-smokers	38	22.3%
Non-smoker	50	29.4%
H/O alcohol intake		
Present	11	6.4%
Absent	88	51.7%
H/O diabetes mellitus		
Present	4	2.3%
Absent	95	55.8%
H/O HIV		
Present	5	2.9%
Absent	94	55.9%
H/O prior treatment		
By private practitioner	69	40.5%
By the Government (DOTS)	30	17.6%

* Total numbers of culture positive patients, *i.e.,* 170.

Table 2. Drug susceptibility pattern of drug resistant patients (*n* = 170)

Item	Number of cases	
	Number	Percentage
Any resistance		
Isoniazid (H)	131	77.9
Rifampicin (R)	116	69
Ethambutol (E)	86	51.1
Streptomycin (S)	93	55.3
Pyrazinamide (Z)	46	27.3
Monoresistance		
Isoniazid (H)	5	2.9
Rifampicin (R)	2	1.1
Ethambutol (E)	3	1.7
Streptomycin (S)	4	2.3
Pyrazinamide (Z)	1	0.5
Multi-drug resistance		
H + R	9	4.7
H + R+Z	2	0.5
H + R + E	14	8.3
H + R + S	26	15.4
H + R + E + S	19	11.3
H + R + E + Z	2	1.1
H + R + S + Z	1	0.5
H + R + E + S + Z	16	9.5
Other patterns		
H + E	2	1.1
H + S	6	3.5
R + Z	6	3.5
R + S	4	2.3
S + Z	3	1.7
E + S	1	0.5
H + E + S	14	8.3
R + E + S	2	1.1
S + E + Z	7	4.1
S + R + Z	4	2.3
S + H + Z	5	2.9
S + R + E +Z	1	0.5

A study from Malawi analyzed data of 748 patients, who had taken the retreatment regimen and of which only 307 (41%) patients had sputum sent for culture and drug sensitivity tests. Fifty-three percent specimens grew organisms resembling *M. tuberculosis*. Of the positive cultures, 81% showed full sensitivity to all drugs tested, 15% showed non-MDR tuberculosis, and 4% showed a MDR tuberculosis pattern (*6*).

Another study from India reported the drug susceptibility profile of 'failures' from the Category-II regimen in pulmonary tuberculosis. Among 431 patients who produced positive cultures, 59% harbored sensitive bacilli, 30% were resistant but non-MDR bacilli, and 11% had MDR tuberculosis (*7*).

Another study from the Netherlands was done to determine acquired drug resistance among failure and relapse cases, in 2,901 patients of smear-positive tuberculosis, who had taken the 2SHRZ/6HE treatment regimen. They concluded that of the failure cases to Category-I regimen (2SHRZ/6EH), 80% had MDR-tuberculosis and among relapse cases, 8% had MDR-tuberculosis (*8*).

Another study, which included data from six countries or areas (Dominican Republic, Hong Kong, Italy, Ivanovo, the Republic of Korea, and Peru), analyzed 6,402 culture positive tuberculosis cases of which, 86% were new cases and 14% were retreatment cases. Among retreatment cases 44.5% were drug resistant and 43.6% had an MDR pattern, while a non-MDR pattern was observed in 56.6% (*12*).

Another study from Morocco was done to evaluate the prevalence and patterns of drug resistance of *M. tuberculosis* isolates from patients with chronic tuberculosis. They showed that 89.3% of the strains were resistant to at least one anti-tuberculosis drug, 5.7% were resistant to only one drug and 83.5% to two or more drugs, with 76.2% of isolates resistant to isoniazid and rifampicin (*13*).

Another study was done among Category-I and retreatment failure patients of pulmonary tuberculosis and they observed MDR tuberculosis, in 1% and 39.7% of cases, respectively (*14*).

Another study from India among 271 new smear and culture positive patients, who had taken Category-I, it was observed that initial drug resistance to any drug was 27% with the MDR pattern being 2.2%. Few studies have reported very high rates of MDR tuberculosis in patients who fail the Category-I regimen. A case-control study from Peru reported that among failures to the Category-I (2EHRZ/4R2H2) treatment, 75% had MDR tuberculosis. Treatment failure in urban Lima has been identified as a strong predictor of MDR tuberculosis (*15*). Another study from India reported a high proportion of MDR tuberculosis strains in both previously untreated (24%) and treatment-failure cases (41%). They also concluded that among new cases, resistance to 3 or 4 drug combinations (amplified drug resistance) including isoniazid (H) and rifampicin (R), was greater (20%) than resistance to H and R alone (4%) at any point in time (*16*).

Another study from India was done in two districts to measure the levels and pattern of resistance to anti-tuberculosis drugs among "newly diagnosed" sputum smear positive pulmonary tuberculosis cases. They further stated that MDR was 0.7% and 3.0%, respectively, in the Mayurbhanj and Hoogli districts (*17*). Another study from India reported a drug susceptibility profile of Category-I failure cases and found that 17% of cases had an MDR pattern, while 30% of cases had a non-MDR pattern (*18*).

The percentage of MDR tuberculosis among supervised Category-II treatment and un-supervised Category-II treatment were 33.4% and 72.4%, respectively.

The present study also highlighted that 63.5% of cases of Category-II failure had not taken DOTS treatment earlier and were treated by private practitioners. In addition to the above, the present study also highlighted the percentage of MDR tuberculosis among DOTS Category-II and non-DOTS Category-II patients were 38.3% and 61.6%, respectively. A study from India also reported that 32% of cases of Category-II failure were from the private sector (*19*).

There is concern regarding the effectiveness of the Category-II regimen for re-treatment cases especially for failures. A study from Siberia showed that only 46% of the patients could be declared cured on the basis of sputum smear microscopy at the end of the therapy (*20*). They also reported that this five drug regimen of Category-II of WHO is inadequate for a re-treatment regimen. The author himself reported a clinical profile of 3 Category-II failure cases earlier and found that 2 of them had an MDR pattern and strongly advocated that Category-II appears good for relapse and treatment for default types of patients. But for the treatment failure group, is this Category adequate (*21*) ? To answer this question a controlled clinical trial is required on a large number of such types of patients.

A major limitation of our study is that sampling is not representative of the general pool of Category-II failure patients in the community. It is a reflection of the drug sensitivity pattern in patients being referred to a tertiary care center; nevertheless we took consecutive samplings over the course of the study.

In conclusion, the present study highlights that more than half of Category-II failure patients have MDR tuberculosis and the RNTCP policy in India of treating all re-treatment cases with the WHO recommended re-treatment regimen (*i.e.,* Category-II) may be adequate except for the MDR tuberculosis patients. Drug susceptibility tests should be done for patients who remain sputum smear positive during the retreatment period and appropriate regimens should be started as early as possible for a better treatment outcome and to reduce transmission of drug resistant tuberculosis.

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(*Received September 29, 2011; Revised February 23, 2012; Re-revised April 30, 2012; Accepted May 15, 2012*)